IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Victor Raso

Application No.: 09/992,994

Filing Date: November 6, 2001

Title: IMMUNOLOGICAL CONTROL OF β-AMYLOID LEVELS *IN VIVO*

Art Unit: 1652

Examiner: Patterson, C.

Docket No.: BBRI-2005

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with The United States Postal Service as First Class Mail in an envelope Addressed to Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on 9/8/04

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DECLARATION UNDER 37 CFR 1.131

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Victor Raso, declare and state as follows:

1. I am an inventor of the invention claimed in U.S. Application No. 09/992,994 filed November 6, 2001, with an effective filing date of June 16, 1999.

2. Prior to June 10, 1999, in laboratories at Boston Biomedical Research Institute (BBRI) in Boston, MA, I, the inventor named in the subject patent application, conceived of the invention of Claims 37-38, 40-42, 43 (as amended), 45, 48-51, 54, and 57-59 of the subject patent application. Documentary evidence that conception occurred before June 10, 1999 is provided in the attached Exhibit A. Exhibit A contains referenced pages from a true copy of a draft of the subject patent application, which was prepared prior to June 10, 1999. The referenced pages, along with the appended correspondence letter, are evidence that conception occurred prior to June 10, 1999. Applicant was diligent from just prior to June 10, 1999 to June 16, 1999, during which final revisions on the draft application were made and transmittal papers were prepared.

Evidence of conception of Claim 37 and 41 can be found on page 6, first full paragraph of Exhibit A. Evidence of conception of Claims 38 and 40 can be found on page 24, last paragraph of Exhibit A. Evidence of conception of Claim 42 can be found on page 6, last paragraph of Exhibit A. Evidence of conception of Claim 43, as amended, can be found on page 18, last paragraph of Exhibit A. Evidence of conception of Claim 45 can be found on page 6, first full paragraph of Exhibit A. Evidence of conception of Claim 48 can be found on page 23, first full paragraph of Exhibit A. Evidence of conception of Claims 49-51 can be found on page 18, first paragraph of Exhibit A. Evidence of conception of Claim 54 can be found on page 6, first full paragraph of Exhibit A. Evidence of Claim 57 can be found on page 6, first full paragraph and page 18, second paragraph of Exhibit A. Evidence of Claim 58 can be found on page 6, first full paragraph and page 18, third paragraph of Exhibit A. Evidence of conception of Claim 59 can be found on page 6, first full paragraph and page 18, last paragraph, also of Exhibit A.

3. The dates and other confidential information have been redacted in the above-referenced Exhibits.

4. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful

false statements may jeopardize the validity of the application or any patent issued thereon.

Signature	Victor Raso
Name	Victor Raso, Ph.D.
Date	8/25/04

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Mouse 1 antiserum	1/1000	2.764

A binding assay was performed to determine whether the monoclonal anti-Aβ antibodies identified ~~by the above assays~~ also bound to the full length Aβ peptides. ^{125}I-Aβ$_{1-43}$ probe was incubated with hybridoma secretions from the indicated clones. A standard polyethylene glycol separation method was used to detect ^{125}I-Aβ$_{1-43}$ bound antibody (Table 2). Results presented in Table 2 indicate that the antibodies generated to the peptide fragments also bound full length Aβ$_{1-43}$.

Table 2 ^{125}I-Aβ$_{1-43}$ Binding Assay

Addition		^{125}I-Aβ$_{1-43}$ Bound (cpm)
Control Hy		3,171
Control Hy		2,903
6E2		15,938
6E2	1/10	9,379
3B1		12,078
3B1	1/10	3,353
8E3		10,789
8E3	1/10	3,249

This means the concentration of the ~~reported result~~ suggests that the ~~albumin~~ bound albumin will interfere w/ anti-body binding.

It was recently reported that when ^{125}I-Aβ$_{1-40}$ is added to human plasma, ~89% binds to albumin (Biere et al., *Journal of Biological Chemistry* 271(51):32916 (1996)). Binding assays were performed in the presence and absence of serum albumin, to determine whether albumin binding ~~would~~ interfere with antibody

binding to Aβ. The ability of purified 5A11 monoclonal anti-Aβ antibody to bind ^{125}I-Aβ_{1-40} was unaffected by the presence of human serum albumin (HSA) at 60 mg/ml, even though this was a 500-fold molar excess over the antibody concentration (Table 3). These results indicate that the ability of antibodies to bind to and sequester Aβ in the blood will not be attenuated by the presence of other binding proteins.

Table 3. ^{125}I-Aβ_{1-40} Binding to Antibody in the Presence of Human Serum Albumin*

Addition	^{125}I-Aβ_{1-40} Bound (cpm)	Specifically Bound (% of total added)
Control	8,560	-
+ 5A11 anti-Aβ	64,589	79
Control + HSA*	3,102	-
+ 5A11 anti-Aβ + HSA*	55,304	75

*HSA at 60 mg/ml (~1 mM); anti-Aβ 5A11 at 2x10^{-6} M; Added ~70,000 cpm of ^{125}I-Aβ_{1-40}

Monoclonal Antibody Production

A mouse was immunized with a KLH conjugate of the central region phenylalanine statine transition state mimic of the central region Aβ_{10-25} peptide. A hybridoma fusion was performed and the resulting monoclonal antibodies analyzed to characterize the specificity of the immune response to the vaccine. Hybridoma supernatants produced in the fusion were screened using ELISA to assess their binding to the Aβ_{1-43} peptide.

The monoclonal antibodies produced were determined to bind to the Aβ_{1-43} peptide adsorbed directly onto an ELISA plate. Strong color reactions were obtained in this ELISA using only 10 μl of hybridoma supernatant while the addition of media alone produced low background color. These results indicate that the

antibodies not only bound to the small peptide immunogen but they were also reactive with the full-length $A\beta_{1-43}$. Importantly, antibodies bound to the carrier-free $A\beta$ peptide adsorbed directly onto microtitre plates, showing their specificity for the peptide rather than the immunogenic carrier. The high affinity 5A11 monoclonal antibody (Table 3) was obtained from this hybridoma fusion. **[VR: THIS IS MISLEADING. PLEASE RECTIFY THIS STATEMENT WITH THE FACT THAT ANTIBODY 5A11 WAS OBTAINED FROM IMMUNIZATION WITH A TRANSITION STATE MIMIC PEPTIDE ANTIGEN.]**

A second mouse was immunized with a KLH conjugate of the $A\beta_{35-43}$ analog encompassing the C-terminal region of $A\beta$. Serum from the mouse was screened for reaction with $A\beta_{1-43}$ adsorbed directly onto the ELISA wells. The assay results are presented in Table 4. The spleen of this mouse was then used for a hybridoma fusion to further characterize the specificity of its immune response. Importantly, none of the mice immunized with $A\beta$ vaccines or the anti-$A\beta$ ascites-producing mice displayed ill effects even though some of those induced antibodies cross-react with mouse $A\beta$ and mouse amyloid precursor protein.

Table 4 ELISA for Binding of Antiserum Directed to the Carboxy-terminal $A\beta$ Peptide

	Antibody Bound (O.D. 450nm)
Addition	Native $A\beta_{1-43}$
Control Serum	0.484
Mouse Antiserum	1.765

Monoclonal antibodies from hybridoma clones generated above were screened for binding to the small carboxy-terminal peptide $A\beta_{35-43}$ and the full-length $A\beta_{1-43}$. Results are presented in Figure 5. The monoclonal antibodies bound to the carboxy-